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# Effects of threonine supplementation on whole-body protein synthesis and plasma metabolites in growing and mature horses

S.L. Mastellar [a,1], A. Moffet [b], P.A. Harris [c], K.L. Urschel [a,*]

[a] Department of Animal and Food Sciences, University of Kentucky, Lexington, KY 40506, USA
[b] Buckeye Nutrition, Dalton, OH 44618, USA
[c] Equine Studies Group, WALTHAM Centre for Pet Nutrition, Melton Mowbray, UK

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ABSTRACT

Current equine threonine requirement estimates do not account for probable use of threonine to maintain gut health and mucin synthesis. The objective of this study was to determine if threonine supplementation (+Thr) would increase whole-body protein synthesis (WBPS) in weanling colts (Study 1) and adult mares (Study 2). Both studies used a crossover design, where each of six animals was studied twice while receiving the isonitrogenous diets. The basal diets contained lower threonine levels (Basal) than the threonine (+Thr) supplemented diets. Threonine intakes in mg/kg BW/day were as follows: 79 (Basal) and 162 (+Thr) for Study 1 and 58 (Basal) and 119 (+Thr) for Study 2, in comparison to the NRC estimated requirements of 81 and 33 mg/kg BW/day for weanling and mature horses, respectively.

Following 5 days of adaptation, blood samples were taken before and 90 min after the morning concentrate meal. The next day, whole-body phenylalanine kinetics were determined using a 2 h primed, constant infusion of [$^{13}$C]sodium bicarbonate followed by a 4 h primed, constant infusion of [1-$^{13}$C]phenylalanine. Most plasma amino acid (AA) concentrations were elevated post-feeding ($P < 0.01$). Lysine and valine plasma concentrations were lower ($P < 0.10$), while methionine, threonine, and glycine plasma concentrations were greater ($P < 0.10$) 90 min post concentrate meal feeding with +Thr in both studies. Phenylalanine flux, intake, oxidation and non-oxidative disposal were similar between treatments ($P > 0.05$). These findings suggest that supplementation of a single AA can affect the metabolism of several AAs and threonine was not a limiting AA in these diets.



## Introduction

Little is known about limiting amino acids in common equine diets or the indispensable amino acid requirements of horses. It has been suggested that the horse's requirements for amino acids may follow the ratio of concentrations of amino acids in the body. Because muscle tissue makes up the majority of the horse as reviewed by Kearns et al. (2002), this has been used to suggest amino acid requirement ratios (Bryden, 1991). To date, there have been no studies to estimate threonine requirements in horses and only estimates based on tissue composition. In some equine diets lysine has been identified as the first limiting amino acid (Hintz et al., 1971; Ott et al., 1981). Threonine (Graham et al., 1994) and methionine (Winsco et al., 2011; Graham-Thiers et al., 2012) have also been suggested as potentially limiting amino acids in yearling and weanling horse diets, respectively.

Supplementation of lysine and threonine together has been shown to increase subjective muscle mass scores and decrease plasma urea nitrogen in mature and aged horses (Graham-Thiers and Kronfeld, 2005). However, in both the Graham et al. (1994) and Graham-Thiers and Kronfeld (2005) studies, which suggested that threonine may be a limiting amino acid, threonine was provided at levels well above the requirement estimated using muscle amino acid composition (Bryden, 1991) in all treatments. The NRC (2007) suggested requirement of threonine for yearlings is 70 mg/kg BW/day, although increased girth circumference was associated with increased threonine intake from 94 to 113 mg/kg BW/day (Graham et al., 1994). Using muscle tissue to estimate requirements does not take into account other uses of amino acids or the fact that muscle protein amino acid composition is not necessarily representative of the composition of other body proteins. For example, the use of threonine by the gastrointestinal tract is considerable in other species.

In the gut, threonine is oxidized and used for mucin production, which varies with diet. Relatively high rates of threonine oxidation in the splanchnic tissues have been demonstrated in piglets (Schaart et al., 2005) and human infants (~70%) (van der Schoor et al., 2007), although this value is less for human adults (~18%) (Chapman

* Corresponding author. Tel.: +1 859 257 7748.
*E-mail address:* klurschel@uky.edu (K.L. Urschel).
[1] Present address: Department of Animal Science, South Dakota State University, Brookings, SD 57007, USA.

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et al., 2013) and adult mini-pigs (~37%) (Remond et al., 2009). Ileal losses of threonine through mucin also contribute to threonine usage in humans (Gaudichon et al., 2002) and intestinal mucin production is considered a major metabolic fate for threonine (Wu, 2009). For rats (Fahim et al., 1987), swine (Fogg et al., 1996), and humans (Roberton et al., 1996), threonine makes up a considerable portion of the mucin proteins. Intestinal mucin synthesis is also influenced by diet constituents, such as fiber (Satchithanandam et al., 1990; Ito et al., 2009). In swine, feedstuffs containing greater amounts of hemicellulose are associated with greater endogenous threonine losses (Myrie et al., 2008). Therefore, increased dietary hemicellulose would be expected to increase threonine requirements in animals. Horses can be fed diets with forages that have a range of acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents, as reviewed by Pagan (1997), but the effect of inclusion of various types of forage in equine diets on threonine use or requirement has not been investigated.

In addition to use for mucin and muscle protein synthesis, other major functions of threonine include immune function, protein phosphorylation, and glycine synthesis, as reviewed by Wu (2009). Glycine is also a precursor for serine synthesis. Threonine supplementation has resulted in greater plasma levels of glycine and serine in pigs (Edmonds and Baker, 1987). Since the metabolism of amino acids is intertwined, threonine supplementation may have consequences beyond increasing protein synthesis.

In a previous study (Tanner et al., 2014), we found that non-oxidative disposal of an indicator amino acid, phenylalanine, was greater in the diet that had a greater content of crude protein [4.1 g/kg bodyweight (BW)/day] and threonine (88 mg/kg BW/day). The phenylalanine non-oxidative disposal results indicated that whole body protein synthesis was limited in the weanlings receiving the diet with lower protein (3.1 g/kg BW/day) and threonine (67 mg/kg BW/day) content (Tanner et al., 2014). Given the amino acid profiles of the diets compared to NRC recommendations, it is possible that threonine was the limiting amino acid in the diet that supported lower levels of whole-body protein synthesis.

As using muscle composition may underestimate threonine requirements and fiber may increase the need for threonine, we investigated in two studies the effect of dietary threonine supplementation to diets, including grass forages, on whole-body protein synthesis in horses. The objective was to determine if threonine supplementation (+Thr) would increase whole-body protein synthesis (WBPS) in weanling colts (Study 1) and adult mares (Study 2) fed typical equine diets. We hypothesized that threonine supplementation would increase WBPS in both studies.

## Materials and methods

### *Animals, housing, and feeding*

#### *Study 1*

All procedures used in this study were approved by the University of Kentucky Institutional Animal Care and Use Committee (IACUC 2012-0991, granted 15 August 2012). Six weanling Thoroughbred colts of similar age (176 ± 30 days) and weight (269 ± 24 kg) were obtained from University of Kentucky's Maine Chance Farm. During both the 7 day adaptation period and days they were not being sampled, the weanlings were kept in individual dry lot pens and were brought into individual sawdust bedded stalls (3.7 m × 3.7 m) twice daily for concentrate feeding. Horses remained in stalls while catheterized on the days that isotope infusions were performed. Bodyweights were taken weekly through the adaptation and study periods on a livestock scale (TI-500, Transcell Technology).

Diets were designed to meet or exceed the 2007 Nutrient Requirements of Horses recommendations (NRC, 2007). Each weanling received each of two diets in a crossover design, which consisted of a concentrate (Buckeye Growth, Buckeye Nutrition) at 1.7% of BW, timothy hay cubes (Premium Timothy Hay Cubes) at 1% BW, and an amino acid supplement. Supplements were isonitrogenous amounts of crystalline threonine (+Thr; 83 mg/kg BW/day) or glutamate (Basal; 103 mg/kg BW/day) top dressed onto the concentrate portion of the feed. The analyzed composition of the diets before the amino acid supplements were added is listed in Table 1. Feed was collected weekly throughout the study and sent to Dairy One Cooperative[1] for proximate analysis and was analyzed for amino acid content as described below.

**Table 1**
As-fed nutrient composition of the feeds used in both diets fed to the weanlings.

| | Timothy hay cubes | Buckeye growth[a] |
|---|---|---|
| Overall nutrient composition[b] | | |
| Dry matter (%) | 91.7 ± 0.6 | 90.7 ± 0.1 |
| DE (Mcal/kg) | 1.78 ± 0.02 | 3.01 ± 0.03 |
| Crude protein (%) | 10.5 ± 0.4 | 19.5 ± 0.1 |
| Lignin (%) | 6.0 ± 0.2 | 2.5 ± 0.4 |
| ADF (%) | 39.1 ± 0.6 | 10.9 ± 1.0 |
| NDF (%) | 57.9 ± 0.9 | 22.7 ± 0.7 |
| Water soluble carbohydrates (%) | 7.0 ± 0.2 | 6.7 ± 0.6 |
| Ethanol soluble carbohydrates (%) | 6.0 ± 0.3 | 5.4 ± 0.3 |
| Starch (%) | 0.5 ± 0.4 | 21.4 ± 0.2 |
| Crude fat (%) | 2.1 ± 0.4 | 8.9 ± 0.2 |
| Calcium (%) | 0.40 ± 0.02 | 0.88 ± 0.10 |
| Phosphorus (%) | 0.21 ± 0.01 | 0.81 ± 0.09 |
| Iron (mg/kg) | 502 ± 79 | 256 ± 24 |
| Zinc (mg/kg) | 89 ± 13 | 180 ± 3 |
| Amino acid composition (g/100 g feed)[b] | | |
| Alanine (%) | 0.44 ± 0.08 | 0.76 ± 0.07 |
| Arginine (%) | 0.54 ± 0.09 | 1.27 ± 0.12 |
| Aspartate + Asparagine (%) | 0.75 ± 0.13 | 1.53 ± 0.15 |
| Glutamate + Glutamine (%) | 0.85 ± 0.14 | 3.32 ± 0.32 |
| Glycine (%) | 0.33 ± 0.06 | 0.67 ± 0.08 |
| Histidine (%) | 0.17 ± 0.03 | 0.36 ± 0.08 |
| Isoleucine (%) | 0.34 ± 0.06 | 0.69 ± 0.10 |
| Leucine (%) | 0.56 ± 0.10 | 1.41 ± 0.30 |
| Lysine (%) | 0.41 ± 0.08 | 0.86 ± 0.10 |
| Methionine (%) | 0.20 ± 0.02 | 0.16 ± 0.01 |
| Phenylalanine (%) | 0.38 ± 0.08 | 0.79 ± 0.09 |
| Proline (%) | 0.47 ± 0.08 | 1.02 ± 0.10 |
| Serine (%) | 0.30 ± 0.06 | 0.65 ± 0.08 |
| Threonine (%) | 0.24 ± 0.04 | 0.36 ± 0.04 |
| Tyrosine (%) | 0.20 ± 0.04 | 0.49 ± 0.04 |
| Valine (%) | 0.41 ± 0.08 | 0.81 ± 0.12 |

[a] Growth Pellet, Buckeye Nutrition, Dalton, OH, USA.
[b] $n = 6$ samples for each feed.

#### *Study 2*

All procedures used in this study were approved by the University of Kentucky Institutional Animal Care and Use Committee (IACUC 2012-0925, granted 1 March 2012). Six mature Thoroughbred mares (12 ± 3 years and 564 ± 23 kg) were obtained from University of Kentucky's Maine Chance Farm. During the 7 day adaptation period and days they were not being sampled, the mares were turned out in grass paddocks in two groups of three with muzzles. Feeding and weighing were performed similarly to Study 1. Mares were brought into stalls to receive individualized meals and overnight.

Each mare on Study 2 received each of two diets in a randomly determined order, which consisted of a low threonine formulated concentrate (Table 2) at 0.8% of BW, chopped timothy (Totally Timothy, Lucerne Farms) at 1.2% of BW, and an amino acid supplement. Supplements were isonitrogenous amounts of threonine (+Thr; 72 mg/kg BW/day) or glutamate (Basal; 89 mg/kg BW/day). The analyzed composition of the diets before the amino acid supplements were added is listed in Table 3. Feed collection and analysis were completed as described for Study 1.

### *Study designs and procedures*

Both studies were conducted as crossover designs, where each horse received both diets in a randomly determined order. All horses received each treatment diet for 5 days prior to sampling with no additional washout between treatments. Growing animals can be studied using phenylalanine kinetics while receiving potentially deficient diets and the risk of detrimental effects associated with prolonged nutrient restriction is minimized due to the short adaptation period (<3 days) required for this method (Moehn et al., 2004b; Elango et al., 2009).

On the morning of day 6 for each treatment venous, blood samples were taken immediately prior to and 90 min post feeding of the morning concentrate meal to investigate the effect of the concentrate meal feeding and amino acid supplements on plasma urea nitrogen and amino acid concentrations.

On day 7, two jugular vein catheters were inserted, one for blood sampling and one for isotope infusion (Urschel et al., 2012). Whole-body phenylalanine kinetics

[1] See: http://dairyone.com/ (accessed 25 September 2015)